S8.P24

Role of DsrC in the mechanism of sulfite reduction by the dissimilatory sulfite reductase DsrAB

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The dissimilatory sulfite reductase (DsrAB) is one of the most important enzymes in the biogeochemical sulfur cycle [1]. It is present in all sulfate reducing microorganisms, in sulfite/thiosulfate/organosulfonate reducers and in sulfur-oxidizing bacteria. This siroheme-containing enzyme catalyzes the reduction of sulfite, but despite years of research, the mechanism and physiological products of sulfite reduction have not been clearly identified. It is not clear why in vitro DsrAB produces a mixture of products including thiosulfate and trithionate, while the closely-related assimilatory enzyme reduces sulfite directly to sulfide. More recently, the reduction of sulfite by DsrAB was proposed to involve also the small protein DsrC, which contains two conserved redoxactive cysteines in a flexible C-terminal arm [2]. The crystal structure of DsrAB in complex with DsrC showed that one of these Cys is located right next to the active site, pointing to the involvement of DsrC in the reduction mechanism [3]. Here, we report recent results from the investigation on the role of DsrC in the reduction of sulfite by DsrAB, using in vivo and in vitro experiments. Our results show that DsrC is directly involved in sulfite reduction and permits the identification of the mechanism and physiological product of this important reaction.

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S8.P25

Dithiothreitol, redox reagent, is affecting proton-translocating ATPase activity of *Rhodobacter sphaeroides*

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The H^+-translocating FoF1-ATPase of purple bacteria, the main membrane-associated enzyme of bioenergetics relevance, can generate proton motive force under various conditions [1]. Its role in photo-fermentation and hydrogen production by these bacteria is suggested [2]. In the current work we have studied effects of DL-dithiothreitol (DTT), a redox reagent reducing disulfides, on N,N′-dicyclihexylcarbodiimide (DCCD)-inhibited ATPase activity of purple bacteria *Rhodobacter sphaeroides* str. MDC6521 (from mineral spring waters in Armenian mountains) membrane vesicles for revealing the regulatory pathways of bacterial redox sensing. The membrane vesicles showed pronounced H^+-translocating ATPase activity, which was inhibited on ~42% by 0.1 mM DCCD. After treatment of membrane vesicles with 0.5–2 mM DTT an additional increase (~1.6–1.8-folds) of DCCD-inhibited ATPase activity was observed. The increase of the ATPase activity by DTT under reducing conditions might be connected with the change of dithiol/disulfide status of this enzyme due to the fact that redox-sensitive thiols gain access to the ATPase. Therefore, the FoF1-ATPase might have an essential role in redox sensing of *R. sphaeroides*.

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Study of the respiratory arsenate reductase from *Halorhodospira halophila* definitively clarifies the evolutionary history of this versatile enzyme

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The three presently known enzymes responsible for arsenic-using bioenergetic processes are arsenite oxidase (Aio), arsenate reductase (Arr) and alternative arsenite oxidase (Arx), all of which are molybdoenzymes from the vast group referred to as the Mo/W-bisPGD enzyme superfamily. Since arsenite is present in substantial amounts in hydrothermal environments (frequently considered as vestiges of primordial biochemistry), arsenite-based bioenergetics has early on been predicted to be ancient. Conflicting scenarios, however, have been put forward proposing either Arr/Arx or Aio as operating in the ancestral metabolism. Phylogenetic data argue in favour of Aio whereas biochemical and physiological data led several authors to propose the Arx/Arr enzyme as the most ancient anaerobic arsenite metabolising enzyme. Here we combine phylogenetic approaches with physiological and biochemical experiments to demonstrate that the Arx/Arr enzyme could not have been functional in the Archaean. We show that Arr reacts with menaquinones to reduce arsenate whereas Arx reacts with ubiquinone to oxidise arsenite, in line with thermodynamic considerations. The phylogeny of the quinone biosynthesis pathway, however, clearly indicates that the ubiquinone pathway is recent. An updated phylogeny of Arr/Arx furthermore indicates a recent emergence of this enzyme. We